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PRODUCTIVE SYSTEM OF THE GOLDEN
HAMSTER (*CRICETUS AURATUS*) AND
THE REACTIVITY OF THIS SYSTEM TO
POSTNATAL HORMONE TREATMENT

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY
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THE EMBRYOLOGICAL DEVELOPMENT OF THE WOLFFIAN AND MÜLLERIAN DUCTS AND THE ACCESSORY REPRODUCTIVE ORGANS OF THE GOLDEN HAMSTER (*CRICETUS AURATUS*)¹

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ONE PLATE (SEVEN FIGURES)

INTRODUCTION

The golden hamster (*Cricetus auratus*) is being widely used in sex research. As has been pointed out by Bond ('45) in her report on care, breeding, and growth of this animal, it offers advantages as a laboratory mammal. It is easy to breed in captivity, is very prolific, and has a short gestation period of less than 16 days.

In spite of its wide use, its embryology has not been thoroughly described. Graves ('43) has studied the early embryology covering the first 9 days post coitum. In his preliminary report, he comes to the conclusion that the hamster develops faster than any other placental mammal, and just as fast as the opossum. In view of this rapidity of development, the embryology of the reproductive system, and especially that of the wolffian and müllerian ducts and the accessory reproductive organs, is of particular interest. Therefore, such a study has been undertaken, mainly as an introduction to the problem of the later development of the reproductive system after birth, and the sensitivity of this system to hormones. Research on the postnatal development and sensitivity of the gonads and accessory organs is already in progress.

MATERIAL AND METHODS

This research is based on data from thirty-two animals, of which twenty-five were embryos and seven were between birth and 6 days of age. The age of the embryos was computed in days and hours after observed copulations. The youngest embryos were 10 days post coitum.

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Four specimens were studied at this age; three at 10 days and 12 hours; eight at 11 days and 19 hours; two each (one male and one female) at the ages of 12 days and 21 hours, 13 days and 20 hours, 14 days and 12 hours, 14 days and 21 hours, 15 days and 12 hours, at birth, and at 5 hours after birth. Two females and one male were studied at 6 days of age. Animals of the same age were littermates with the exception of a few cases.

Embryos were removed from the mother, freed from their membranes, and fixed in Bouin's fluid. The whole embryo was sectioned in the younger ages, but only the posterior half or the urogenital region in older specimens. All the material was cut serially at a thickness of 10μ and stained in Harris haematoxylin. The length of the wolffian and müllerian ducts, as well as that of the accessory reproductive organs, was computed by counting the sections in which these structures appeared and multiplying by the thickness of the sections.

The main emphasis of this study was placed on the development of the wolffian and müllerian ducts, and the accessory reproductive organs — prostate, seminal vesicles, coagulating glands, bulbo-urethral glands. The gonads were observed only superficially, from the point of view of sex differentiation.

OBSERVATIONS

Ten days post coitum. A study of the hamster embryo at 10 days post coitum reveals the reproductive system in a very primitive condition. It consists of an accumulation of epithelial cells forming a very small and indistinct genital ridge, which is beginning to separate by a shallow groove from the mesonephric fold. There is no müllerian duct present at this age. The wolffian duct, however, being part of the primitive urinary system, is already well developed, and has a lumen. It runs posteriorly to the cloaca, which it joins, although the junction is solid.

The mesonephros is very small and is located ventral and medial to the post-cardinal vein in the urogenital fold. It has very few tubules, most of which are still solid.

The permanent urinary system is beginning to develop at this stage. The ureter is already branching off from the wolffian duct. It is a small hollow outpocket $150\text{--}170\mu$ long, running dorsal and cranial from its point of origin in the mesonephric duct. There is some condensation of tissue around the ureter which might be interpreted as the metanephric blastema.

Ten days and 12 hours post coitum. At the second age in this series, the gonad primordium is a little more advanced in its development. The genital ridge is more distinct, its division from the mesonephric fold being marked by a deeper groove. There is also a mass of cells forming a dark staining epithelial nucleus in the region where the sex cords will later develop. The müllerian duct is still completely absent. The wolffian duct has grown longer during this 12-hour period; however, its stage of development is not more advanced than at the earlier stage, and it is still solid where it joins the urogenital sinus.

The ureter seems to be growing very fast during these early stages. In 12 hours it has grown more than three times in length in some cases. Its lumen is also larger.

The cloaca is now almost completely divided into rectum and urogenital sinus.

Eleven days and 19 hours post coitum. A great advance in the development of the urogenital system has taken place since the last stage. The gonad has not only grown tremendously in size, but it is also beginning to differentiate sexually. Sex can be determined microscopically. As figures 1 and 3 show, the cells in the outside layers of the gonad in some embryos are beginning to elongate and assume the shape of tunica cells. There is also some tendency of the epithelial mass to organize into small cell clusters, thus suggesting the very beginnings of testis cords. On the basis of these findings, three of eight embryos were considered males, and the others females or undifferentiated as to sex. The indifferent gonad or ovary (fig. 2) consists of an epithelial mass surrounded by the germinal epithelium, but it has no tunica.

It is interesting to note that in these differentiating males the solid efferent ducts of the mesonephros are already sending downgrowths into the gonad, which connect with the solid rete, and so form the urogenital connection. In the female or undifferentiated gonad, however, this connection is not very clear in most cases.

Though the gonads at this age are beginning to differentiate, the ducts do not show any difference in the two sexes. As can be seen in figures 1 and 2, the wolffian duct is as well developed in the sexually undifferentiated embryo as it is in the differentiating male. It has grown about as much in one as in the other. It is patent throughout, but still joins the urogenital sinus without a lumen at the junction. The müllerian duct is now present for the first time. It is shorter and narrower than the wolffian duct. Its anterior end has an ostium about 50 μ in length on the ventral side of the mesonephric fold. The duct follows

antero-posteriorly as a narrow solid cord in some regions, though in others it is beginning to acquire a small lumen. It is located at the outer tip of the mesonephric fold. At this age it has not developed any farther posterior than the region of the gonads. There is no visible difference between the müllerian duct of the differentiating male and that of the undifferentiated or female embryo (see figs. 1, 2).

The wolffian body has also increased in size since the last age. It has more tubules and they all have lumina. Most of these tubules join the wolffian duct.

The metanephros has developed greatly in the last 30 hours. It is a fairly large body in the region of the mesonephric fold, and lies medial to it. It has many hollow branching tubules. The ureter is very long and is still attached to the wolffian duct just before the latter joins the urogenital sinus. This junction is still solid.

Twelve days and 21 hours post coitum. Sexual differentiation has advanced rapidly. Sexes can be detected very readily by microscopic examination of the gonad. The tunica albuginea of the testis is now a very distinct band, several layers thick, of lighter staining elongated cells between the germinal epithelium and the epithelial mass. The cell clusters in the epithelial mass are beginning to round up gradually into testis cords, but these are not definitely distinguishable as such until 24 hours later. The epithelial mass of the ovary is also rounding up into cell clusters, which are separated from each other by connective tissue strands. The connection between efferent tubules and rete cords is still more marked in the testis than in the ovary.

The wolffian duct of the female is growing and developing as fast as in the male. It is larger in diameter than in the previous age, and has a large lumen. In both sexes it joins posteriorly the solid sulcus prostaticus.

As already mentioned in the previous age, the müllerian duct grows antero-posteriorly. This caudal growth has now progressed greatly, so that at this age the duct has reached the posterior end of the embryo and has the same length as the wolffian duct. Posterior to the gonads, the two mesonephric folds have now grown together in both sexes, and continue posteriorly along the midplane as the genital cord. As a result, the müllerian ducts come to lie side by side in the center of the cord, while the wolffian ducts appear lateral to them. The müllerian duct is exactly the same length in both sexes; however, it is beginning to show differences in the two sexes. In the male, this duct is already degenerating. This is detectable by the condition of its lumen, which

is present in only one-third of its length, while in the female a lumen is present throughout.

Posteriorly, the müllerian duct in both sexes seems to join the wolffian duct on each side before the latter joins the urogenital sinus. If this is the case, the wolffian duct widens tremendously and becomes solid for some distance anterior to the urogenital sinus. However, it is more likely that these wide solid structures joined by the müllerian ducts are not the widened wolffian ducts, but two solid dorsal projections of the urogenital sinus, known as the prostatic sulci, which the wolffian duct has already joined.

The bladder is now formed and the ureters join it, but this junction is still solid.

Thirteen days and 20 hours post coitum. The testis cords, which have been gradually developing, are now definitely formed. There is a marked contrast between the tissue forming the cords and the interstitial tissue between them. The ovary does not show any further development, except for a more marked cluster arrangement of cells in the epithelial mass. The rete and the efferent ducts of the testis are developing very slowly and show no conspicuous further differentiation at this age. In the female, however, the rete seems more developed and its connection with mesonephric tubules more marked than at earlier stages.

The wolffian duct is now beginning to show sex differences. It is longer and more developed in the male. In the female it is degenerating; is absent at intervals along its course; and is much smaller in size than the müllerian duct.

The müllerian duct, which had started degenerating in the male 24 hours earlier, shows further degeneration. Its anterior end is disappearing and its lumen is now obliterated except in the region of the genital cord. In this region, the right and left ducts come to lie side by side, as explained above. In the female the müllerian duct is very well developed. In both sexes this duct still seems to join the wolffian duct on each side before reaching the urogenital sinus. However, the same explanation holds here as in the previous age, that the solid structure it joins may not be the expanded end of the wolffian duct, but the solid sulcus prostaticus of the urogenital sinus.

At this age the primordia of the accessory reproductive structures are beginning to develop. The first ones to appear are those of the bulbo-urethral glands. In both sexes they emerge from the posterior region of the urethra as two small solid buds (40 μ in length).

Fourteen days and 12 hours post coitum. The testis has now descended to the region of the bladder. The ovary has also descended and is now just posterior to the kidney. The testis cords, as well as the sex cords of the ovary, have advanced very little since the last age. In the male, the wolffian duct is very well developed, while in the female, degeneration has proceeded roughly antero-posteriorly at great speed, so that only three pieces remain. The first is a solid remnant of the right duct about 170μ long directly posterior to the gonads. The others are remnants of both right and left ducts (230μ long) in the extreme posterior end. They have lumina and still join the prostatic sulci.

The müllerian duct in the male is also degenerating antero-posteriorly, as was shown in the preceding age. In this specimen, however, a solid remnant of 200μ appears in the region of the gonad on each side. In the genital cord patent sections of 580μ are present. In the female, the utero-vaginal canal has not formed, although the right and left ducts are in close contact. In both sexes the müllerian duct joins the solid sulcus prostaticus medially and posterior to the junction of the wolffian duct.

The buds of the Cowper's (or male bulbo-urethral) glands at $14\frac{1}{2}$ days are still solid, and they appear to be six times as long as the ones at the preceding age. There is a condensation of tissue around each bud of the gland, the significance of which is not clear. In the female, the Bartholin's glands, the homologues of the Cowper's glands, degenerate almost immediately. In the previous age they were exactly the same size as those of the male; 16 hours later their presence is very doubtful, and thereafter, they are definitely absent.

Another interesting point in embryos at this age is the appearance for the first time of the anlage of the seminal vesicles in the male, with no homologue in the female. These glands are outpockets of the wolffian duct in the region of the genital cord. They grow in a dorsal and cranial direction and are lined with the same tall columnar epithelium of the wolffian ducts. The wolffian duct in the male is very well developed at the site of formation of the seminal vesicles. In the female, however, it is degenerating antero-posteriorly and is already absent in the region where the seminal vesicles would develop. Therefore, no homologues of such glands appear.

The mesonephros at this age is disappearing and only very few inconspicuous tubules are left. The ureters have lumina at their junction with the bladder.

Fourteen days and 21 hours post coitum. The sex cords and the rete in the testis and ovary do not show any further conspicuous development. Moreover, the degeneration of the ducts in opposite sexes has not gone any farther at this age than at the previous age.

There is some further development, however, in the accessory reproductive glands. The anlage of the Cowper's gland has now a very distinct duct coming from the urethra. Their homologue in the female is absent. The first anlagen of the prostrate gland in the male appear at this age. One dorsal and one ventral prostate bud $70\ \mu$ long emerge from the margin of each sulcus prostaticus. As shown in figure 4, they are very small, round, solid buds. Their homologue is not present in the female at this age.

Fifteen days and 12 hours post coitum. The degeneration of the wolffian duct in the female has progressed farther posterior, so that only $70\ \mu$ are left at the extreme posterior end. The müllerian duct in the male has not degenerated any further than in the previous age. It is more definite at this age that both ducts join the solid sulcus prostaticus separately, the junction of the wolffian duct being anterior to that of the müllerian duct.

The Cowper's gland has now a well developed duct which measures $130\ \mu$. The seminal vesicles have not grown in length but they seem to have a larger lumen. A pair of small solid buds can be recognized extending cranially from the ventral side of the sulcus prostaticus into the genital cord, at the point where the wolffian duct joins the sulcus. These seem to be the coagulating gland anlagen. The prostate gland has now a second pair of solid dorsal buds posterior to the first pair, and of the same length — $30\ \mu$. The ventral buds appear as in the previous age. In the female, however, there is still no indication of a prostate gland.

Birth (15 days and 16 hours post coitum). The embryos studied at birth had a rather short gestation period as compared to the mean found by Bond ('45) of 15 days and 21 hours. The condition of the testis and ovary does not indicate any further marked development of the cords. The rete is solid in both sexes, and in the female its connection with the efferent ducts is still not very conspicuous, though it seems to be present. The ovary is now completely surrounded by the ovarian capsule.

The ducts show no further degeneration in the opposite sex than in the previous age. In the male the remnants of the müllerian ducts form a uterus masculinus at intervals, then separate again into right and left tubes. In the female, the two müllerian ducts have not definitely

fused to form the utero-vaginal canal, but the partition between them is degenerating.

The accessory reproductive glands, however, show some further development. Around the solid anlage of the Cowper's gland there is an accumulation of tissue the significance of which is not understood. The seminal vesicles have grown to $400\ \mu$ in length, but they still appear as simple unbranched outpockets of the wolffian ducts. The coagulating gland anlagen have grown cranially and are beginning to acquire a small lumen. Now they appear to join the urogenital sinus dorsally and slightly anterior to the point where the wolffian and müllerian ducts join it. The prostate gland in the male has now two pairs of dorsal buds ($30\text{--}80\ \mu$), and two pairs of lateral buds. The first pair in each case is anterior to the second. There is only one pair of ventral buds, $30\ \mu$ long. They are all very small and solid. Prostate anlagen appear now for the first time in the female. There are three small, solid ventral buds emerging from the urethra. The first bud occurs only on the left; posterior to this is a pair.

Five hours after birth. The rete testis is now beginning to hollow. In the female, the rete is solid and its connection with the efferent ducts is no more obvious than in previous ages. The remnant of the wolffian duct in the female is very small in diameter and has no lumen for most of its length. It is short and discontinuous and is not present at the posterior end. In the male there is a piece of the uterus maculinus, which joins the urogenital sinus. As shown in figure 5, the utero-vaginal canal is now well formed in the female.

In the Cowper's glands the formation of a lumen has progressed from the duct into the anlage of the gland itself. The accumulation of tissue around the gland primordium is still very obvious. This thickening of tissue is also present in the female, but no anlage of the Bartholin's gland is present. There is also some accumulation of tissue around the seminal vesicles. These are still unbranched and have not grown significantly since the last stage. There is a very short ejaculatory duct ($80\ \mu$ long).

The primordium of the coagulating gland appears thicker and more conspicuous, but it is about the same length as before. It still does not have a lumen throughout, but its connection with the sinus is now hollow.

The prostate gland anlagen are still very small, solid, and inconspicuous. The ventral buds of the male have not grown any larger, but there is now a thickening of tissue around each ventral bud. Anterior

to these ventral buds there is only one pair of very small, solid lateral buds about $50\ \mu$ in length. These are located in the region where the seminal vesicles branch off from the wolffian duct. Posterior to the ventral buds are the dorsal buds. There is only one pair of these and though they are also very small, they are the largest ones at this age ($100\ \mu$ long). In the female there is only one pair of ventral prostate buds (fig. 5). They are about the same length, but they extend farther out from the sinus and seem to be larger in diameter than those of the male.

An interesting observation is that in the region of the ovary a remnant of the mesonephros is still present and extends for $400\ \mu$.

Six days after birth. Male. At 6 days after birth, the testis still has solid tubules. However, a very conspicuous change has taken place since the last age. The nuclei in the tubules are now gradually assuming a peripheral arrangement, thus leaving a clear center of cytoplasm. The rete testis is very large and has a large lumen. It connects with numerous efferent ducts, which in turn empty into a very coiled ductus epididymis, and this into a well developed vas deferens, with a wide lumen. Emerging as an outpocket from the vas is the ampullary gland. It grows cranial to its point of origin. The seminal vesicles have already emerged at a point posterior to this. Their growth within the first 6 days after birth is very marked. They have not only increased greatly in length, but also in complexity. They are slightly coiled anteriorly and their lumen is branching. The ejaculatory duct is very short — $80\ \mu$.

The coagulating gland is moving slightly dorsolateral in its growth, so that at this age it is already growing close to the seminal vesicles. It is now completely hollow. The Cowper's gland is large and convoluted, and has a wide lumen.

The prostate gland grows tremendously during the first 6 days. It is now a large gland around the urethra and is arranged into four lobes. As seen in figure 6, the two dorsolateral lobes are much larger than the two ventral lobes. The left dorsolateral lobe in this specimen appears posterior to the other three. The ducts in all the lobes run posteriorly to join the urethra. The dorsolateral lobes have multiple ducts, which join the urethra dorsally or laterally for some distance. The ventral lobes also have multiple ducts. The first three ducts in these lobes join the urethra laterally, instead of ventrally, with the exception of one on the right side. The rest of the ducts, posterior to these, all join ventrally. Posterior to the four lobes of the prostate, there is a series of very small buds, most of which are solid. They are arranged around

the urethra at different levels in a dorsal, lateral, and ventral position. Their size and stage of development can be compared with the condition of the first buds of the male prostate (fig. 4). Whether these small posterior buds are prostate buds or buds of the urethral glands is difficult to determine.

There is a remnant of the müllerian duct in the form of a uterus masculinus which is about $110\ \mu$ long and has rather a wide lumen.

Female. In the ovary at 6 days the cells of the sex cords are undergoing rapid mitosis. The rete ovarii is solid. Connecting with it is the epoöphoron, which has a lumen and is $80\ \mu$ long. There is also a very distinct paroöphoron with a lumen and a total length of $320\ \mu$. It appears anterior to the ovary, but since it does not connect with the rete, it cannot be considered epoöphoron. Its anterior position is probably due to the rotation of the ovary. These mesonephric tubules connect with each other in some cases, but they do not all open into a common duct, therefore no trace of the wolffian duct is recognizable.

At this age the vagina can be distinguished from the uterus by its wider lumen. At its anterior end it has developed two lateral pouches on its ventral side which connect with the vagina by two narrow ducts. These are the vaginal pouches, which characterize the hamster female. In the adult, they appear at the extreme posterior end of the vagina.

The prostate gland in the female also grows a great deal during the first 6 days after birth. The anlagen occur around the urethra in the form of dorsal, lateral, and ventral buds (fig. 7). These buds extend through a length of $1080\ \mu$ along the urethra, but they do not form lobes as in the male. There are over forty buds of different sizes ranging from 30 – $240\ \mu$ in length. The larger buds are hollowed through their whole length, others are solid buds with hollow ducts joining the urethra, and the smallest are completely solid. Some are straight, others coil. The ducts all run posteriorly to join the urethra. There is no trace of the Bartholin's gland.

RESUMÉ

From this account, the development of the reproductive system of the hamster can be reviewed as follows:

Male. The first indication of a genital ridge occurs at 10 days post coitum, and becomes more definite 12 hours later when it has acquired an epithelial nucleus. The gonad forms soon after this age, and by 11 days and 19 hours, some gonads are differentiating into testes as shown by the beginning of the tunica layer. Two days later, at 13 days and

20 hours, the testis cords are definitely present. These cords remain solid through embryonic life, and though they are still solid at 6 days of age, their nuclei are already arranged in the periphery of the cord. The rete testis is solid until after birth, but its connection with some of the mesonephric tubules is established very early at the time of the beginning of sex differentiation.

The mesonephric duct is complete and has a large lumen by 10 days post coitum. At this early age it has already formed the ureters, as its first pair of outpockets. The male wolffian duct shows no superiority over the female until 13 days and 20 hours. From this age until birth it appears more prominent in the male. At 14½ days it gives off its second pair of outpockets — the seminal vesicles. At birth and shortly after, the male mesonephric duct begins to grow tremendously in length and has a very wide lumen. The epididymis is differentiated from the vas as a long coiled region of the duct by 6 days of age. At this age the third pair of outpockets has formed from the vas as the ampullary glands.

The müllerian duct is not formed until the time of the beginning of sex differentiation, at 11 days and 19 hours. Twenty-six hours later, at 12 days and 21 hours, it begins to show signs of degeneration in the male. This degeneration is antero-posterior and continues rather rapidly, though it shows a great deal of individual variation. While some embryos at 13 days and 20 hours show anterior degeneration of this duct, others at 14½ days still retain a large solid piece in the region of the gonads. The uterus masculinus formed by the fusion of the right and left müllerian ducts is present at birth and 6 days after.

The first accessory reproductive glands to develop in the male are the Cowper's glands. The anlagen are present at 13 days and 20 hours. These glands show a very marked growth for the first 16 hours, but after this their growth in length is very slow until after birth. The second glands to develop in the male are the seminal vesicles at 14½ days. At 14 days and 21 hours the first buds of the prostate gland are formed (fig. 4). Few new buds appear at the next ages. The greatest advance in the development of this gland takes place between birth and 6 days. At 15½ days the coagulating gland anlage is formed in the male. This gland develops rather slowly until after birth.

Female. At the time of the beginning of sex differentiation, 11 days and 19 hours, the female is not yet easily recognizable from the undifferentiated stage, so that embryos which are not definitely males can be either females or undifferentiated. Not until 12 days and 21 hours can

the ovary be recognized as such. At this same age the sex cords in the ovary are beginning to appear. These cords develop very slowly so that there is hardly any visible difference at older ages. In the embryo studied at 12 days and 21 hours a rather distinct band of cells is seen between the germinal epithelium and the epithelial mass. This was interpreted as a primary cortex, but its complete absence at older ages makes this interpretation doubtful. At birth the ovary is already surrounded by the ovarian capsule.

The rete ovarii remains solid throughout development. Though solid, its connection with the mesonephric tubules is obvious. This connection is not clearly visible until 12 days and 21 hours, that is, about 26 hours later than in the male.

The wolffian duct in the female begins to degenerate at 13 days and 20 hours, 1 day after the ovary can definitely be recognized. This degeneration proceeds roughly antero-posteriorly. However, in some embryos this duct is degenerating at irregular intervals along its length, while in others the posterior end piece is missing when there are still some remnants anteriorly. In the specimen studied at birth there is a piece of the right and left duct 80μ long at the extreme posterior end, still joining the urogenital sinus. Five hours after birth there is an anterior remnant of this duct on each side, and no posterior piece remains. Six days after birth, however, these remnants have completely disappeared, leaving no trace of this duct in the female.

The müllerian ducts do not fuse to form the utero-vaginal canal until after birth, though the partition between the ducts is very thin for about 20 hours before. At 5 hours after birth, the canal is definitely present for the first time (fig. 5). Six days after birth the vagina is distinguishable from the uterus and has the two lateral vaginal pouches.

The only male accessory glands which have homologues in the female are the Cowper's and the prostate. The Cowper's homologue, or Bartholin's gland in the female, develops first, and at the same age as in the male — 13 days and 20 hours. However, it is very short lived, since 16 hours later its presence is doubtful, and in 24 hours it is definitely absent. The prostate gland, however, persists for a longer time. It is first present at birth, which is approximately 20 hours after it appears in the male. Between birth and 6 days it undergoes great development.

DISCUSSION

The golden hamster has a very short gestation period compared to the 21- to 22-day-period in the rat and the 19-day-period in the mouse.

Bond ('45) has reported a mean period of gestation of 15 days and 21 hours, which confirms the findings of previous authors (Bruce and Hindle, '34; and others). The short gestation brings about a very rapid embryonic development. This has already been reported by Graves ('43) in relation to the early embryonic stages of this animal. From the preceding description of the development of its reproductive system, the rapidity of development is again very obvious. The very first indications of a genital ridge are present at 10 days post coitum, which means that the whole prenatal development of the reproductive system takes place in about 6 days. This is very much faster than in the rat or mouse. In the rat, the first indications of a genital ridge appear at 12 days and 18 hours (Price, unpublished), or about 9–10 days before birth. In the mouse (Brambell, '27 a) they appear at 9 days post coitum, that is, 10 days before birth. So, while the reproductive system of the rat has a prenatal development of 9 days and the mouse, of 10 days, that of the hamster is only 6 days. Such a discrepancy in length of time in the development of this system might lead us to expect a very primitive and undeveloped condition at birth, comparable to that of the opossum. However, such is decidedly not the case, since, except for the prostate gland, the genital system is, in general, no more primitive at birth in the hamster than in the rat.

Recognizable sex differences in the hamster appear less than 2 days after the first appearance of the genital ridge. In the rat (Price, '35, '36; Moore and Price, '42) visible male differentiation occurs 2 days after the genital ridge begins to develop, and in the mouse (Brambell, '27 a) sex differences are not detectable until $2\frac{1}{2}$ to 3 days after the ridge is present. Though development is a little faster in the hamster, the plan of sex differentiation corresponds closely to that of the other two animals. The first apparent differences in the sexes are seen in the gonads, the testis differentiating before the ovary. The ducts do not differentiate sexually until much later. It is interesting to observe that, as in the mouse (Brambell, '27b), the connection between the efferent tubules and the rete of the male gonad is established before that of the female. In fact, in the hamster this connection is present as soon as the testis begins to differentiate — 11 days and 19 hours.

The wolffian body and duct are completely established in the mouse before the reproductive system begins to develop, at 9 days post coitum (Brambell, '27 b). In the rat, they are already well developed by 11 days and 18 hours post coitum, that is, 24 hours before the beginning of the reproductive system (Price, '35). In the hamster, though the duct is

well developed by the time the genital ridge appears, the wolffian body has not developed very far yet but continues to grow and form more tubules for at least 2 days later. The degeneration of the wolffian duct in the female begins 2 days after sex differentiation is apparent in the gonad. This is exactly the case in the rat (Price, '35; Moore and Price, '42) and the mouse (Brambell, '27 b). Degeneration proceeds antero-posteriorly in the three rodents, though in both the rat and the hamster slight departures from this antero-posterior procedure are quite common. This degeneration occurs faster in the hamster. That is, the whole life span of the wolffian duct in the female rat after sex can be determined is about 6 days, since the last remnant of it is seen at 20½ days post coitum (Price, '35). In the hamster it lasts approximately 4 days. There is still a small piece left in the hamster 5 hours after birth, but its condition is so degenerate that it is doubtful that it would persist much longer. Other remnants of the wolffian system in the female hamster are the epoöphoron and the paroöphoron. They are still present at 6 days of age. In the rat, Price ('35) does not report any paroöphoron, but she found that the epoöphoron is present at 5 days of age. In the mouse, Brambell ('27b) found that the paroöphoron, degenerates early and cannot be detected at birth. The epoöphoron, however, persists throughout life.

The müllerian duct begins to develop in the hamster at the time that sexual differentiation is first apparent in the gonad. In the rat and the mouse, however, this duct is present somewhat in advance of the stage when the testis can be identified (Price, '35; Brambell, '27 b). In the hamster this duct grows very rapidly toward the posterior end, and attains its entire length in 1 day. Degeneration of the müllerian duct in the male starts very early. While the posterior end is still growing, the anterior portion is already losing its lumen, and so showing signs of degeneration. In the mouse (Brambell, '27 b) and the rat (Price, '35; Moore and Price, '42), degeneration of this duct in the male also begins anteriorly while the duct is still growing posteriorly. The condition of this duct in the male at birth and 6 days of age corresponds closely in the hamster and the rat.

Therefore, as can be seen from the above discussion of the development and degeneration of the wolffian and müllerian ducts in the rat, the mouse, and the hamster, the three animals agree remarkably closely.

In regard to the development of the accessory reproductive glands, the hamster also follows the pattern of the rat with some modifications. The order in which these glands begin to develop does not correspond

exactly. That is, in the hamster the Cowper's gland anlage appears first, and very soon after sex can be recognized. Both the male and female have the anlage of the gland, but, while in the male it grows and develops rapidly, in the female it disappears almost immediately. This is not so in the rat, since, according to Price ('35), it develops steadily in the female up to the first day of life. The second anlage to appear in the hamster is the seminal vesicle. This is the first in the rat, according to Price ('36). In all other details this gland develops similarly in both animals and reaches a comparable stage of development at birth and 6 days after. The fact that the seminal vesicle does not develop at all in the female rat is explained by Price ('36) by stating that this region of the wolffian duct in the female is already "missing or incomplete" before the time of development of these glands. This can be offered as an explanation also in the hamster, since the female wolffian duct is missing in the region where the seminal vesicles would be expected to develop.

The prostate gland is the next accessory gland to appear in the hamster. It has only about 1 day of prenatal life, during which its development is very slow. In the rat (Price, '36), it has about 2 days of prenatal life and has a very rapid development during this time. Consequently, the condition of the gland at birth in the male hamster is very much simpler and more undeveloped than in the rat. However, the condition at 6 days corresponds very closely with that of the rat at 5 days of age. A comparison of the prostate of a 6-day-old hamster (fig. 6) with that of the 5-day-old rat (fig. 5, Price, '36) shows very close similarity in degree of development. In regard to morphology of the gland, however, it is also obvious from these two figures that in the hamster the two ventral lobes are definitely separated from each other, but in the rat they are almost fused, forming one ventral lobe. This separation of the ventral lobes in the hamster persists throughout life.

The female prostate gland in the rat begins its development at the same age as the male (Mahoney, '40), or shortly after (Price, '36). It corresponds to only a portion of the ventro-lateral lobes of the male. The buds grow and coil slightly up to 5 days of age, according to Price. In the hamster, the female prostate does not appear until birth, that is, 1 day after it first appears in the male. Growth and development is also slight, but the condition at 6 days (fig. 7) shows a fairly well developed gland with both dorso-lateral and ventral buds. The buds are distributed through a long section of the urethra (1080 μ). Thus far, this gland has not been found in the adult female hamster. However, in the

rat well developed prostate glands are found in some adult females (Marx, '31, '32; Witschi, Mahoney, and Riley, '38; Price, '39).

Such a comparison of the hamster with the rat and the mouse brings us to the conclusion that, except for minor details, the development of the reproductive system of these three rodents corresponds remarkably closely. While the period of gestation of the hamster is about 6 days shorter than that of the rat, and 3 days shorter than that of the mouse, the speed of development is such that at the time of birth the stage of development of the reproductive system is essentially the same.

SUMMARY AND CONCLUSIONS

The embryology of the reproductive tract of the golden hamster was studied in thirty-two specimens, from 10 days post coitum to 6 days after birth. Emphasis was laid on the wolffian and müllerian ducts, and the accessory reproductive glands. The development of these structures was compared to that of the rat and mouse. The following conclusions are drawn:

1. The reproductive system develops faster in the hamster than in the rat and mouse.
2. The genital ridge is visible at 10 days post coitum.
3. The testis is recognizable at 11 days and 19 hours; the ovary 24 hours later.
4. The müllerian duct in the male begins to degenerate at 12 days and 21 hours, while the wolffian duct in the female starts degeneration 24 hours later.
5. The prostate gland begins to develop at 14 days and 21 hours in the male. It has a slow prenatal growth. In the female the prostate begins at birth. By 6 days it is quite well developed.
6. The seminal vesicles first appear at 14½ days; the coagulating glands 1 day later. These glands have no homologues in the female.
7. The bulbo-urethral glands begin to develop at 13 days and 20 hours in both sexes; they disappear soon in the female.
8. In general, the reproductive tract of the golden hamster at birth is as well developed as that of the rat.

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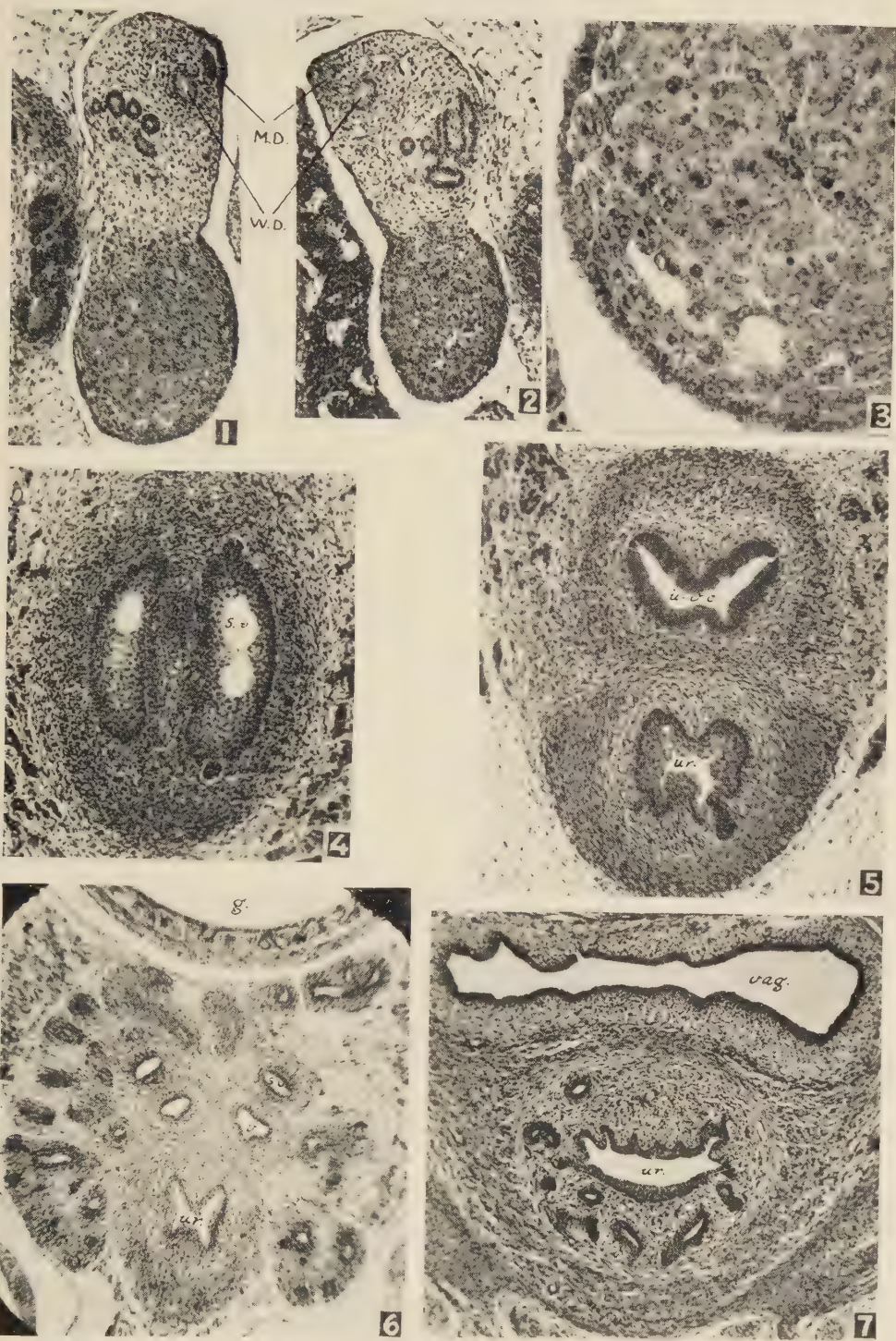
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PLATE 1

EXPLANATION OF FIGURES

- 1 Differentiating testis of a hamster embryo 11 days and 19 hours post coitum. MD, müllerian duct; WD, wolffian duct. $\times 86$.
- 2 Undifferentiated gonad, or ovary, of a hamster embryo 11 days and 19 hours post coitum. MD, müllerian duct; WD, wolffian duct. $\times 86$.
- 3 High power view of a portion of figure 1, to show developing tunica layer. $\times 309$.
- 4 Section through prostatic sulci of a male hamster embryo 14 days and 21 hours post coitum, to show the earliest buds of the prostate gland on the dorsal and ventral side of the sulcus. sp, prostatic sulcus. $\times 82$.
- 5 Section through the genital cord and urethra of a female hamster 5 hours after birth, showing the utero-vaginal canal (u-vc), and ventral prostate buds emerging from the urethra (ur). $\times 82$.
- 6 Section through the region of the prostate gland of a male hamster 6 days after birth. g, gut; sv, seminal vesicle; v, vas; ur, urethra. $\times 42$.
- 7 Section through the vagina (vag.) and urethra (ur.) of a female hamster 6 days after birth showing prostatic anlagen around the urethra. $\times 65$.



THE POSTNATAL DEVELOPMENT OF THE REPRODUCTIVE SYSTEM OF THE GOLDEN HAMSTER (*CRICETUS AURATUS*) AND ITS REACTIVITY TO HORMONES¹

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IN A previous study on the embryological development of the golden hamster (Ortiz, 1945), it was found that the reproductive system develops more rapidly in the hamster than in the rat or mouse. It was observed that, in spite of the much shorter gestation period (16 days), the reproductive tract of the golden hamster is as well developed at birth in most respects as that of the rat. In the present work the postnatal development of the gonads and accessory reproductive organs has been studied with especial reference to the rate of development and the age at which the organs are capable of responding to exogenous and endogenous hormone stimulation. Price and Ortiz (1944) concluded from their study of reactivity in the post-

natal reproductive tract of the albino rat that the capacity to respond to hormone stimulation develops gradually. It is of particular interest to correlate the age and rate of development with the degree of hormone sensitivity in the hamster and to compare these factors in the prepuberal hamster and rat. For this purpose the present work has been undertaken.

MATERIAL AND METHODS

This research is based on the results obtained from 546 hamsters from the same stock as those used in the embryological study. Of these, 285 were injected, and 261 were kept as normal controls. A summary of normal body weights and organ weights of all the animals used for controls at each age appears in Table 1. With some exceptions, each litter was divided into approximately the same number of injected and control animals. Table 2 summarizes the number of animals in each injection series.

The hamsters were injected subcutaneously for 6 days with daily doses of 10 r.u. of equine gonadotropin, 0.1 mg. of testosterone propionate, or 1 r.u. (0.166 μ gm.) of estradiol benzoate. The sex hormones were dissolved in sesame oil, and

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TABLE 1
SUMMARY OF NORMAL WEIGHTS

AGE IN DAYS	FEMALES					MALES					
	No. of Animals	Body Weight (Gm.)	Organ Weight (Mg.)			No. of Animals	Body Weight (Gm.)	Organ Weight (Mg.)			
			Ov.*	Ovd.	Ut.			Testis	V.P.	S.V.	C.G.
6.....	25	5.5	0.81	5.5		28	5.4	8.3			
10.....	27	8.6	1.2	1.1	9.0	31	9.1	19.3	0.3	1.3	0.4
16.....	22	17.5	2.1	3.9	18.0	22	17.1	51.3	1.0	2.8	0.6
26.....	26	40.5	14.6	7.0	56.2	22	37.2	290.0	5.6	13.6	2.7
36.....	26	46.1	17.4	10.1	132.3	24	43.0	663.3	12.3	44.0	7.8
56.....	6	83.0	20.7	17.1	244.5						

* Ov. = ovary; Ovd. = oviduct; Ut. = uterus; V.P. = ventral prostate; S.V. = seminal vesicle; C.G. = coagulating gland.

TABLE 2
SUMMARY OF RESPONSE OF ORGANS IN AVERAGE
PERCENTAGE CHANGE IN WEIGHT

TREATMENT	AUT. AGE IN DAYS	NO. OF ANIMALS	CON-TROLS			TREATED			Ov.*	OVD.	UT.	TESTIS	V.P.	S.V.	C.G.
			♀ ♀	♂ ♂		♀ ♀	♂ ♂								
Equine gonadotropin (10 r.u. daily for 6 days)	6	48	8	12	11	17	—	15†		0		35†			
	10	44	11	9	14	10	—	15†	18	—	3	38	19	75	14
	16	26	5	7	7	7	—	138	106	516	80	143	156	353	
	26	32	10	6	9	7	—	123	111	344	56	75	155	116	
	36	41	7	11	9	14	—	809	75	195	4	42	157	135	
	56	14	6		8		—	549	36	120					
Total.....		205	47	45	58	55									
Testosterone propi- onate (0.1 mg. dai- ly for 6 days)	6	26	8	6	5	7	—	10		42		—	2		
	10	40	7	11	9	13	—	12	137	79	—	11†	10	78	50
	16	34	7	8	10	9	—	12	24	106	—	17	116	180	202
	26	30	9	7	8	6	—	32	—	31	—	38	67	71	100
	36	31	9	6	9	7	—	22	—	24	—	42	19	20	62
Total.....		161	40	38	41	42									
Estradiol benzoate (1 r.u. daily for 6 days)	6	37	10	10	6	11	—	48		346		8†			
	10	40	9	11	8	12	—	9	177	227	—	31†		—	6
	16	35	11	7	10	7	—	3	55	146	—	29	—	68	0
	26	36	7	9	11	9	—	15	—	18	6	—	22	—	5
	36	32	10	7	8	7	—	4	—	14	—	37	—	46	—
Total.....		180	47	44	43	46									
Grand total.....		546	134	127	142	143									

* Abbreviations same as in Table 1.

† Analyzed statistically—not significant.

‡ Analyzed statistically—highly significant.

the equine gonadotropin was aqueous. Injections in the three groups were started at birth and at 4, 10, 20, and 30 days of age. Animals were killed about 24 hours after the last injection, at the ages of 6, 10, 16, 26, and 36 days. In addition to these animals, a small group of females was injected with equine gonadotropin for 6 days at from 50 to 56 days of age, to study the changes in reactivity of the ovary and uterus at this age.

The time of the opening of the vagina was observed, and smears were studied in some injected and control females. The size of the clitoris and penis of injected animals was observed at autopsy and compared to these organs in the normal controls.

At autopsy the ovaries, oviducts, uteri, testes, ventral prostates, coagulating glands, and seminal vesicles were dissected rapidly to avoid drying of the tissues and were weighed on a torsion balance. The two ovaries, oviducts, testes, seminal vesicles, or coagulating glands of each animal were weighed together. At 6 days the organs were so small that the oviduct and uterus were dissected and weighed together and the male accessory organs were left attached to the urethra and sectioned together; but weights were found to be unsatisfactory. All tissues were fixed in Bouin's fluid. The ovaries, oviducts, and the anterior portion of the uteri were sectioned serially at 10 μ ; samples of the male organs were sectioned at 6 μ . The tissue was stained with Harris' haematoxylin. Some duplicate samples of the male accessory organs were stained with Ehrlich's haematoxylin or modified Mallory's triple stain.

Tissues from 154 animals were studied microscopically. Measurements of the diameter of testis tubules and ovarian follicles were taken in some cases. No tissues were studied in the 56-day-old females.

The mean body weights and organ weights of all injected animals at a given age in each series were compared with those of the corresponding control animals. The percentage differences in weight are given in Table 2. In some cases in which this percentage was of doubtful significance, Fisher's formula was applied, and the *t*-value for significance was obtained. Body weight was not affected by any of the hormones injected at any age.

NORMAL POSTNATAL DEVELOPMENT OF THE REPRODUCTIVE SYSTEM

AT 6 DAYS OF AGE

At 6 days after birth the ovary is a very small organ, with a mean weight of 0.8 mg., and is in a very simple stage of development. The primordial sex cells are arranged in clusters of irregular size and shape, which occupy mainly the outer region of the gonad. These clusters are surrounded by smaller, slightly elongated epithelial cells. The oviduct is also very simple, and the epithelium is low. The uterus is undeveloped, small in diameter, and has a low epithelium and a small, simple lumen (Fig. 4).

The testis has solid tubules, which average 46 μ in diameter. They are formed of one row of small basal epithelial cells arranged around the periphery inside the basement membrane; many cells are undergoing mitosis. The center of the tubules is packed with large, light-staining cells, in which mitoses are not observed. The stroma between the tubules consists of a large number of two kinds of cells: spindle-shaped mesenchymal cells around the outside of the basement membrane of the tubule, as well as elsewhere in the intertubular spaces; and round interstitial cells, larger than the mesenchymal cells, arranged in clumps or loose in the intertubular space.

The male accessory reproductive organs are still in the anlage stage at 6 days. The ventral prostate consists of two small lobes composed of buds from the urethra. These are hollow at the proximal end but solid at the distal tip. The seminal vesicle is a short, hollow, saclike structure, coiled anteriorly, with a branching lumen and a low epithelium. The coagulating gland was not studied histologically at this age.

AT 10 DAYS OF AGE

By 10 days of age the ovary has increased somewhat in weight, although it is still very small. It is completely filled with small follicles, but around the periphery the "egg-cluster" condition typical of the 6-day ovary is still present. The larger follicles have a mean diameter of about $53\ \mu$ and in most cases consist of one layer of follicle cells. In some follicles, however, there is very active mitosis taking place, so that a second layer is already forming and in others there are several layers. Glands are just beginning to develop in the uterus at this age. They appear as very small indentations in the uterine epithelium. The vagina is open in all females with a body weight of 8 gm. or more. The vaginal smear is scanty and consists mainly of leucocytes and debris.

The testis is now over twice as heavy as it is at 6 days, but the solid tubules have not increased very much in diameter. However, a second row of basal epithelial cells is beginning to appear inside the first row, and the large cells in the center of the tubules are less numerous and their nuclei have become vacuolated. The ventral prostate is larger than it is at 6 days. The acini are larger in diameter, although they are still solid at the tip. The epithelium is low. The seminal vesicle has a moderately high pseudostratified epithelium with elongated nuclei crowded together, and many cells

show mitoses. The coagulating gland has, for the most part, small, solid acini, although a few of the acini have lumina.

AT 16 DAYS OF AGE

The ovary has a mean weight of 2.1 mg., and the largest follicles are almost twice as large as they are at 10 days. These follicles occupy the center, while very small follicles are located toward the periphery of the ovary. There are also more follicles containing several layers of follicle cells than there are at the previous age, and very few have only one layer. Follicles having a multiple number of ova are very numerous at 16 days and remain quite numerous for some time after this. These polyovular follicles may contain 2-6 ova, which, in most cases, are separated from each other by rows of future granulosa cells. The epithelium of the oviduct is much higher than at 10 days. The nuclei are basal, and the cytoplasm is vacuolated. The epithelium of the uterus is now folded, and the elongated nuclei occupy most of the cell. The uterine glands have grown markedly since the last age. They are much more numerous, and some are beginning to form lumina. The vaginal smear is no longer scanty in some females at this age, and it consists of many cornified cells, leucocytes, and nucleated epithelial cells, plus some atypical material which may be classified as debris. In some cases all three cell types are present; in others, two types are found.

The testis has grown tremendously and has a mean weight of 51.3 mg. The tubules are larger in diameter than at the previous age, and some of them are beginning to acquire a small lumen. There are several rows of small epithelial cells. Many of these cells, bounding the small lumen, are dark, and their nuclei appear to be either dividing or degenerating. According to Hargitt (1926), in the rat

these are degenerating spermatogonia. This is probably the case in the hamster also, since at 26 days the number of cells in the tubule is considerably reduced. By 16 days all the large cells in the center of the tubule have disappeared. In the spaces between the tubules, the round interstitial cells appear less numerous than at 10 days of age.

The ventral prostate, although still very light in weight, is greatly developed as compared to the previous age. It has many large and highly convoluted acini. The epithelium is high and folded and so-called "light" areas are present in the cells above the basal nuclei. These light areas are very similar to the ones described in the ventral prostate of the rat (Moore, Price, and Gallagher, 1930). At this age they are still rather small and indefinite, since they are just beginning to develop. The seminal vesicle has doubled in weight since 10 days, but it does not appear much more developed. The coagulating gland is approximately the same size and has the same differentiation as before.

AT 26 DAYS OF AGE

At 26 days of age, hamsters are extremely variable in size, the body weights ranging from 22 to 52 gm. The weight of the ovary, as well as its degree of differentiation, is also variable. Ovarian weight ranges from 10 to 20.6 mg., with a mean weight of 14.6 mg. (Table 1). In general, the smaller animals have the lighter and less well-developed ovaries, although exceptions to this condition are numerous. The simpler ovaries have many rather small follicles, about 100–150 μ in diameter and without antra, while the heavier ovaries have many follicles of 200–385 μ in diameter and with antra. Polyovular follicles are abundant in all the ovaries examined in

26-day-old hamsters. In one ovary, 130 polyovular follicles were found. Most of these follicles have two ova, although some have three or four. Corpora lutea are completely absent in ovaries at this age, which shows that ovulation has not yet occurred, even in the largest animals.

The uterus is much larger than it is at 16 days and is extremely variable. Its weight ranges from 16.4 to 134 mg., and there seems to be little or no correlation between uterine size and the weight of the animal. Uterine glands are very numerous and branching. They extend through the endometrium, almost to the muscle layer. The larger uteri have a very folded mucosa and a high, pseudostratified epithelium, while the smaller uteri have a simple mucosa with a lower epithelium.

The testes have increased markedly in weight since 16 days of age and have already descended into the scrotal sac in some animals, although in others they are still in the inguinal canal. All the tubules have lumina, but the lumina are larger in the tubules of heavier testes. Tubules with the larger lumina have a very few of the small epithelial cells. There are many primary spermatocytes in the process of being exfoliated into the cavity. Interstitial tissue is very sparse.

The ventral prostate has large, distended acini, with high epithelium. The light areas are more marked than they are at 16 days. The seminal vesicle is very much larger and more highly developed. The epithelium is extremely convoluted, although perhaps lower than that of the ventral prostate. Above the nucleus there is a round or oval light spot, comparable to the light area in the ventral prostate but smaller. There is evidence of secretion in the acini. The coagulating gland is now very large and well developed. It has highly convoluted acini with high epithelium, round basal

nuclei, and clear cytoplasm. The acini seem to be filled with secretion.

AT 36 DAYS OF AGE

Hamsters have grown little in size since the previous age and are still variable in weight. The ovaries are also variable and have increased little in weight since 26 days. However, ovulation has now taken place, as evidenced by the presence of several corpora lutea in most of the ovaries studied and ova in the oviducts in a few cases. Polyovular follicles seem to be disintegrating, since they are found now in small numbers. The uterus is still variable in size. The epithelium is columnar, with vacuolated cytoplasm. The uterine glands appear to be at the same stage of development as at 26 days. The vaginal smear shows that cycles have begun in these animals.

Vaginal smears were studied in a group of nine females belonging to two litters. Examination of smears was started at the age of 24 days, and smears were taken daily for 10 days until the animals were 33 days of age. At 31 days all females had smears composed almost exclusively of a huge mass of nucleated epithelial cells. This, according to investigators (Peczenik, 1942; Kupperman, Greenblatt, and Hair, 1944; and Ward, 1946) who have studied the vaginal smear of the hamster, is the typical smear picture on the morning after ovulation has occurred.

The testes, although still variable in size, are now very much larger than they are at 26 days and have descended into the scrotal sac in all animals. The tubules are larger (100–150 μ) in diameter, and most of them have a wide lumen. They have many spermatogonia, spermatocytes, and sperm heads, although the latter are not present in the smaller testes. Interstitial cells are very sparse. The light areas in the epithelial cells of the

ventral prostate and the seminal vesicle are sharper and more definite than at previous ages. Figure 3 shows these light areas in the seminal vesicle. The coagulating gland does not seem to have developed a great deal since 26 days of age.

SUMMARY OF NORMAL DEVELOPMENT

The normal development of the reproductive tract of the golden hamster can be summarized as follows: The ovary of the hamster grows very slowly during the first 16 days after birth (Table 1). Ovarian growth is suddenly accelerated between 16 and 26 days and is slow again during the next 20 days. At 6 days of age the condition of this organ is extremely primitive, and it consists of clusters of primary sex cells surrounded by epithelial cells. Follicles are formed by 10 days, some of which grow steadily and acquire antra by 26 days of age, while others degenerate. The first ovulation has occurred by 36 days, when corpora lutea are first found in the ovary.

The uterus grows much faster than the ovary, but it also has a sudden acceleration of growth after 16 days. Uterine glands first appear at 10 days, acquire lumina at 16 days, and are fully developed by 26 days of age. The vagina normally opens when the females reach a weight of about 8 gm. This usually occurs around 10 days of age. The vaginal smear is very scanty at first and contains mainly leucocytes and some debris. Cornified and nucleated epithelial cells soon appear. Around 30 days of age, cycles have started.

The testes grow very rapidly during the first 36 days of life, but the greatest increase in weight occurs between 16 and 26 days. By 26 days they are beginning to descend, and at 36 they are in the scrotal sac. At 6 days of age the testis is quite primitive in structure. It has solid

tubules with one row of basal epithelial cells. A very small lumen is beginning to appear in the tubules at 16 days; it gradually increases in size until 36 days of age, when it has reached its maximum diameter. Sperm heads appear at about 36 days. Interstitial tissue is most abundant at 6 and 10 days, after which it appears to decrease and is very sparse at 26 and 36 days.

The male accessory organs are extremely small and simple in structure during the first 2 weeks of life. There is a sudden acceleration of growth and development between 16 and 26 days. At 16 days of age the ventral prostate acquires lumina in all the acini, and light areas appear in the epithelium. These, however, are not fully developed until much later in life. The seminal vesicle acquires similar light areas in the epithelium at 26 days, but they are round or oval and smaller than in the epithelial cells of the ventral prostate. The coagulating gland has a high epithelium and round basal nuclei.

EFFECT OF HORMONE INJECTIONS

I. EQUINE GONADOTROPIN

AT 6 DAYS OF AGE

It was very difficult to accumulate enough data on animals injected from birth to 6 days of age because the mother hamster has a tendency to destroy her young if she is disturbed in any way soon after littering. Moreover, marking the treated animals by cutting off the ear or the tip of the tail was very unsatisfactory, since the bleeding was a great factor in stimulating the mother to destroy the litter. Tying the tip of the tail with fine silk proved to be a very adequate marking system, but this method was not used until the experiments were nearly completed. In an effort to avoid marking, some entire litters were injected, leaving

no littermates for control animals, while other litters were killed at 6 days to serve as normal controls. This was done in the gonadotropic series in one-half of the cases in the females and in about one-third of the cases in the males. This procedure was extremely unsatisfactory, since our colony of hamsters has not been closely inbred and litters differ greatly as to body weight and weight of the reproductive organs.

At 6 days of age there is a 15 per cent reduction in weight of the ovary (Table 2), which is not statistically significant. No histological changes from the normal can be observed in the ovaries. The oviduct and uterus of the injected females are not affected in weight or histology. Therefore, it can be concluded from these results that the injection of equine gonadotropin from birth to 6 days has no detectable effect on the reproductive system of the female hamster.

The 35 per cent increase in the testis weight is statistically significant and has a *t*-value of 4.2, with a probability of $< .001$. This percentage increase represents a consistent increase in testis weight in every single animal injected with equine gonadotropin when it is compared with its uninjected littermates. Histological studies, however, show no difference between the testes of the injected and the uninjected males. There are no histological changes in the accessory organs of the male.

AT 10 DAYS OF AGE

Injections of the gonadotropic hormone produce an increase in the ovary of 15 per cent, with a *t*-value of 4.2, which represents a probability of $< .001$. The diameter of the follicles is increased from 53 to 77 μ . The oviduct weight is increased 18 per cent, and histological changes from the normal are observed. The epithelium appears greatly stimu-

lated; the cells are higher, and there are more mitoses than in the epithelium of the oviduct of the control females. Although the uterus is not increased consistently in weight, there is some evidence of histological stimulation. The epithelium seems higher, and there are more gland crypts in the uterus of the injected animal. In one particular case the uterus is exceptionally increased in weight and shows tremendous stimulation. This uterus weighs 32.4 mg., while the mean uterine weight of the injected animals is 7.94 mg. Its diameter is greatly enlarged, and the epithelium is much higher and folded. The uterine glands are larger and more numerous and with lumina beginning to form. The muscle layer is also better developed.

The testis is stimulated in its growth to about the same degree as in the 6-day-old males. As before, the testes of all the injected males are consistently heavier than those of the noninjected animals, so that, in spite of the absence of histological differences, the testes of hamsters at 10 days are definitely stimulated by this gonadotropic agent. The ventral prostate is increased 19 per cent in weight, but it shows no histological changes from the normal. The seminal vesicles are increased 75 per cent in weight. This represents a consistent increase in all injected animals. In spite of the simplicity of the seminal vesicle at this age, its lumina are larger, and the epithelium is much higher than in the control. The coagulating gland is increased in weight 14 per cent over the control, but no histological changes are observed. The penis is enlarged in some injected animals.

AT 16 DAYS OF AGE

The ovary has increased 138 per cent in weight (Table 2), and the follicular diameter and the theca seem to be slight-

ly enlarged by the gonadotropic injection. The oviduct is increased 106 per cent over that of the normal animal, and it has a larger diameter and a greatly stimulated epithelium. The uterus reaches its maximum increase in weight over the normal at this age in the gonadotropic series, and it shows great histological stimulation. The diameter is greatly enlarged, the epithelium more folded and very much higher, appearing pseudo-stratified, and the cells are vacuolated.

The epithelium in the uterine glands is also vacuolated, the glands are greatly enlarged and have much larger lumina than the glands of the normal animal. The clitoris in some injected females is enlarged and seems swollen and wider than the clitoris in normal females.

The testis is now increased 80 per cent over the normal weight for this age. There is also an increase in tubular diameter of from 64 to 82 μ . Lumina are present in all tubules of the testes of injected males and are larger than the lumina of normal testis tubules at this age. The ventral prostate has now reached its maximum percentage increase in weight and has more acini than the normal gland. The epithelium is more folded but not any higher, and there are light areas in the gland of the normal, as well as of the injected, animals. The seminal vesicle has also reached its maximum weight increase, and the histology of this gland in the injected male shows great stimulation over that of the control. The epithelium is much higher and folded and has precocious light areas. These are pale and small as compared to the light areas of the older glands. The coagulating gland also reaches its peak of reactivity at this age, with a weight increase of 353 per cent over the normal weight. The epithelium is very much stimulated; it is folded and much higher in the gland of

the injected animal. The penis is enlarged at this age.

AT 26 DAYS OF AGE

The ovary is increased in weight to about the same degree as it is at 16 days, but it has large follicles, 230–400 μ in diameter and with antra. Although the normal littermates are of approximately the same body weight as the injected females, their ovaries have follicles below 150 μ in diameter and without antra. There is some thecal and interstitial luteinization in the ovaries of the injected females. The oviduct is enlarged 111 per cent but is not affected histologically. The uterus is increased 344 per cent over that of the control. The epithelium is more folded; the cells are tall and columnar with small, round, basal nuclei and vacuolated cytoplasm. The uterine glands are not very numerous, but more of them have lumina than the normal uterus. The muscular layer is more prominent. The epithelium of the normal uterus is lower and has elongated nuclei, and the cytoplasm of the cells is not vacuolated.

The testis is not increased so much over the normal weight as it is at 16 days; however, gonadotropic stimulation is evidenced by an increase in average tubular diameter of from 87.5 to 112 μ . There is also some indication of an increase in interstitial tissue. The ventral prostate is increased 75 per cent in weight, the seminal vesicles 155 per cent, and the coagulating gland 116 per cent. However, no histological changes are found.

AT 36 DAYS OF AGE

At this age the ovary reaches its peak of reactivity, as shown by the weight increase of 809 per cent (Table 2) and by the histological picture. The weight of the ovary is increased from a mean of 17.4 mg. in the controls to 146.4 mg. in

the injected animals. Figure 1 shows the degree of stimulation of the follicles when compared with Figure 2, the ovary of a normal littermate female. Most follicles in the ovary are enlarged tremendously and have huge antra. These large follicles give the ovary a mulberry appearance when examined macroscopically. Many blood points are present; more than twelve have been observed in one ovary. Some thecal luteinization is found, but there are no corpora lutea. The oviduct is increased 75 per cent but shows no histological changes from the normal. The uterus is increased 195 per cent in weight. Its diameter is enlarged, the mucosa is much more folded, and the epithelium is higher than in the uterus of the normal animal. It is difficult to determine whether the clitoris is enlarged in females at this age.

The weight of the testis is not affected at 36 days. Interstitial tissue still appears more abundant in the testis of the injected male. The ventral prostate is increased 42 per cent in weight over that of the control but is not affected histologically. The seminal vesicle is increased in weight to about the same degree as in the last two ages. Acini are more distended than in the normal gland, and the epithelium is more folded. The coagulating gland is increased 135 per cent in weight, but its histology is not affected. The penis is enlarged in only a few animals; in most it is difficult to determine.

AT 56 DAYS OF AGE

In view of the very high reactivity of the ovary at 36 days, some females were injected daily at from 50 to 65 days of age in order to determine the ovarian sensitivity at an older age. These females show a decrease in reactivity of the ovary to gonadotropic stimulation. The percentage increase in ovarian weight of injected animals has dropped from 809 per cent

at 36 days to 549 per cent at 56 days. Although no histological study was made of the tissue at this age, macroscopic observation of the ovary shows extreme follicular growth, a great number of hemorrhagic follicles, and some corpora lutea. This gives the ovary the mulberry appearance already observed at 36 days of age. The reactivity of the oviduct and uterus is also lower than at the previous age. The weight increase of the oviduct is now 36 per cent and that of the uterus 120 per cent.

II. TESTOSTERONE PROPIONATE

AT 6 DAYS OF AGE

It was even more difficult to gather enough data on animals injected from birth to 6 days with the sex hormones than it was with equine gonadotropin because of the oily medium of the sex hormones. Even when no seepage of the oil occurred at the time of the injection, the animals acquired the odor of the oil, which was probably a disturbing factor for the mother. This caused the destruction of so many litters that the amount of data obtained is inadequate. Because of the small number of cases, the percentage weight changes were not analyzed statistically.

The ovary at this age ranges in weight from 0.4 to 1.0 mg., with a mean of 0.8 mg. in the control, and from 0.4 to 1.4 mg., with a mean of 0.72 mg., in the injected animals. There are no histological differences between the ovaries of the injected and the control animals. In the female accessory organs, however, the percentage increase in weight over the normal is very marked and is probably significant. It represents an increase of from 6.2 to 8.8 mg. This increase is quite consistent in all the injected animals and is accompanied by very marked histological changes. The oviduct has a much higher epithelium; the uterus is greatly

increased in diameter, and the epithelium is folded and higher than in the uterus of the normal animal. Uterine glands are produced precociously by direct stimulation, and, although they are not very numerous, they are very conspicuous and some are beginning to acquire lumina. This precocious development of uterine glands is very similar to that produced with estradiol benzoate (Fig. 4). The clitoris is greatly enlarged and is wider and longer than in the normal female.

The weight of the testis is not affected at this age, and there are no detectable histological differences between the testes and accessory reproductive organs of the injected and the control males. The penis is greatly enlarged.

AT 10 DAYS OF AGE

Reduction in the weight of the ovary at this age is again of doubtful significance because of the small number of cases. It represents a weight change from a mean of 1.08–0.96 mg., and there is not a consistent decrease in all the injected animals. There are no changes in the histology of the ovary of injected females at this age or at any of the subsequent ages. At 10 days the oviduct reaches its highest percentage increase in weight, which is much above that of the next age. It is greatly stimulated and shows great histological changes. The epithelium is extremely high and somewhat pseudostratified in places. The uterus is increased 79 per cent in weight over the control. The diameter is greatly enlarged, and the epithelium is more folded than in the control animal. The smooth-muscle layer is wider and appears more distinct. Uterine glands are highly stimulated; they are very large and have acquired a lumen. The vaginal smear is not affected by male hormone at this age. The clitoris is greatly enlarged.

The 11 per cent reduction in the weight of the testes is highly significant and has a *t*-value of 4.8, although reductions are not found in all the injected animals. There are no changes in the histological picture. The ventral prostate is increased only 10 per cent in weight, but the gland appears more developed and has more acini with lumina than the gland of the control male. Some acini have a high epithelium, although no light areas are produced precociously by the male hormone. The seminal vesicle is increased 78 per cent, and its epithelium is higher and more folded. The coagulating gland is also stimulated and has a weight increase of 50 per cent, but no histological differences are apparent. The penis is enlarged.

AT 16 DAYS OF AGE

The ovary is reduced in weight to the same degree as at the previous age, and its histology is not affected. The oviduct is stimulated much less at this age than it is at 10 days. Its weight increase is only 24 per cent, and the epithelium shows less indication of stimulation, although it is still higher than is the oviduct of the control females. The uterus is stimulated more at 16 days than it is at earlier ages both in weight increase and in uterine gland growth. The clitoris in some of the injected females is larger than the penis of the normal 16-day-old male.

The testis is reduced 17 per cent in weight but shows no histological changes as compared to the control. The ventral prostate is increased 116 per cent, reaching at this age its maximum percentage weight increase with male hormone. The acini are larger, but the epithelium is not higher than in the normal. Light areas are present in the prostate of both injected and control animals and are not larger or more conspicuous in the gland

of the injected animal. The seminal vesicle is increased 180 per cent, which also represents a peak of reactivity. The epithelium is a little higher in the treated males; and small, precocious light areas are produced. The coagulating gland also reaches its maximum weight increase at this age. It is increased 202 per cent and appears more developed than in the untreated male. The acini are larger and more numerous, and the epithelium is higher. All injected males show a remarkable increase in the size of the penis.

AT 26 DAYS OF AGE

By 26 days the ovary is reduced 32 per cent in weight. This represents a reduction in mean weight of from 14.6 (range: 10.2–18.0 mg.) to 9.8 mg. (range: 8.2–10.8 mg.). The ovarian weight is consistently reduced in all injected animals, when compared to littermate, as well as to nonlittermate control females. There are no histological changes in the ovary. The oviduct is reduced in weight by 31 per cent, but its histology is not affected. The uterus is reduced 38 per cent. The normal uterus is so variable in weight (16.4–134.0 mg.) and in histology in the 26-day-old females that it is very difficult to determine whether this reduction is really due to the male hormone. The clitoris of injected females is as large as the penis of the normal male of the same age.

The testis is reduced 67 per cent in weight and shows inhibition of tubule growth in diameter and of lumen formation. The average diameter of the tubules is reduced from 84 to 65 μ . While most tubules in the testes of control males have either a large lumen or a small one beginning to form, there are almost no tubules with lumina in the testes of the injected males. The ventral prostate is not increased in weight as much as it is at the previous age. The acini are greatly

distended in the gland of the injected male, but the epithelium is not higher. The increase in weight of the seminal vesicle is now only 100 per cent, and the coagulating gland has an increase of only 26 per cent. There are no histological changes from the normal in either gland. The penis is still enlarged by the injection of testosterone propionate.

AT 36 DAYS OF AGE

The ovary shows a reduction of 22 per cent in weight, accompanied by no histological changes. The accessory female organs are reduced to about the same degree as in the previous age and show no detectable histological changes from the normal.

The testis is reduced 19 per cent in weight, and there are no histological differences from the normal. Sperm heads are found in the testes of both normal and injected males. The ventral prostate is increased 62 per cent and has some acini that are much more distended than in the normal gland. The seminal vesicle is not so enlarged as it is in the previous age and is not affected histologically. The coagulating gland is enlarged 44 per cent in weight and shows no histological difference from the controls. The penis is still enlarged by the injection.

III. ESTRADIOL BENZOATE

AT 6 DAYS OF AGE

There is a 45 per cent reduction in the weight of the ovary at 6 days of age. This represents a reduction in mean weight of from 0.5 to 0.26 mg. The significance of this reduction was not determined because of the small number of cases. The oviduct and uterus, however, are so tremendously increased in size in every injected female that, in spite of the few cases, it is unquestionable that these organs are really stimulated by the injection of female hormone. Table 2 shows a

weight increase of 346 per cent of oviduct and uterus together. This is an increase in mean weight of from 5.1 to 22.8 mg. Histologically, the oviduct shows an extremely high epithelium, with vacuolated cytoplasm. The uterus is very much enlarged in diameter, the endometrium and the lumen are larger, and the epithelium is higher and folded. Uterine glands are precociously developed; some are in the crypt stage, but others are much larger and more developed and are beginning to acquire a lumen (see Figs. 4 and 5).

The injection of female hormone opened the vagina in all the females in this series, even though their body weight range was between 5.3 and 6.3 gm., which is very much below the weight at which the vagina normally opens in the hamster. The smear taken at 6 days of age in these injected females was very abundant and consisted of either a definite mass of nucleated epithelial and cornified cells or a combination of cornified cells, leucocytes, and epithelial cells. The clitoris is wider and longer than in the normal animals, and the whole genital region appears swollen.

There is no significant weight change in the testis of animals 6 days of age, and its histology is not affected. No histological differences are observed in any of the accessory organs of the treated, as compared to the untreated males. The penis is also unaffected.

AT 10 DAYS OF AGE

The ovary is not affected in weight or in histology. The oviduct and uterus are stimulated to about the same degree as they are at 6 days, as determined by the percentage increase of their combined weights. The uterine glands of the injected animals are now long and coiled and have large lumina. They seem to be more numerous than in the normal animal. The vagina is open, as before, in all

injected animals. The smear on the tenth day consists of a mass of cornified cells. The clitoris is enlarged and the genital region highly swollen.

The testis is reduced 31 per cent in weight, which is highly significant (t -value = 5.1). No histological changes are observed. The ventral prostate is reduced so much in size that it was impossible to dissect it from the urethra, so it was fixed *in situ*, and no weights were obtained. It does not differ histologically from the normal gland at this age. The seminal vesicles and the coagulating gland were dissected, weighed, and sectioned together. There is no significant reduction in their weights and no histological change from the normal. The penis is now reduced in size.

AT 16 DAYS OF AGE

The ovary is not affected; the oviduct is increased only 55 per cent in weight, and it is difficult to detect any histological difference between the oviduct of the injected and the control females. The uterine increase of 146 per cent is less than it is at 10 days. Histologically, however, the degree of stimulation seems about as great as it is at the previous age. The uterine glands are larger, more numerous, and have larger lumina than the glands of the normal uterus. The vaginal smear of the injected females is very abundant and consists of either a large mass of cornified cells and a few nucleated epithelial cells or a mass of both cell types in approximately equal proportions. The clitoris is enlarged, but no swelling of the genital region is observed.

The testis is reduced about the same amount in weight as it is at the previous age and shows no histological differences from the normal. The ventral prostate is reduced 68 per cent, and there is a reduction in epithelial height and an inhibition of light areas which are normally

present in the ventral prostate at this age. The seminal vesicle is not affected in weight or in histology. The coagulating gland is reduced 15 per cent in weight but does not differ histologically from the normal. The penis is again reduced in size.

AT 26 DAYS OF AGE

The ovary is reduced 15 per cent in weight, the oviduct 18 per cent, while the uterus is increased 6 per cent. In view of the small number of cases, it is very likely that the reduction in the ovary and the oviduct and the slight increase in the uterus have little or no significance. This is especially true in the case of the uterus, which has already been described as extremely variable in weight at this age and at 36 days. The vaginal smear in the injected female is difficult to distinguish from that of a normal animal. It is doubtful if the clitoris is enlarged.

The testis is reduced 22 per cent, and there is some slight indication of reduction of tubular diameter and inhibition in the formation of lumina, but this is not consistent in all the treated animals. The male accessory organs and the penis are unaffected.

AT 36 DAYS OF AGE

There is no effect on the weight of the ovary, but there is some indication of inhibition of follicular growth, although it is questionable. The reduction in weight in the female accessory organs is probably merely apparent, because of the great variation in the normal organs. In addition, all normal females now have cycles, so that the effect of the injection may depend upon the stage of the cycle at which treatment is begun. The clitoris and the vaginal smear are not affected.

The testis is more reduced in weight by the injection of female hormone than at any other age, and this reduction is consistent in the testes of all the injected

males. There is again some inhibition of the growth of the lumen in the tubules, although it is still doubtful because of the inadequate number of cases. All the male accessory organs are reduced in weight, and their reduction is consistent in all cases. The penis is not affected.

In summary, the effect of hormone injection in the golden hamster during the first 36 days of life can be reviewed as follows:

Equine gonadotropin.—Gonadotropic stimulation of the ovary occurs for the first time at 10 days. At this age there is evidence from the histological study of the uterus and the oviduct that the ovary is stimulated to increase its production of female hormone. There is also a significant increase in growth of the ovary as a whole. The reactivity of the female gonad is suddenly increased to a peak at 36 days. At 56 days, reactivity is still very high but lower than at 36 days. Histologically, gonadotropic injections enlarge the size of the follicles at 10 and 16 days and the thecal layers at 16 days. At 26 days, follicular growth and formation of antra are stimulated, but the highest degree of follicular stimulation occurs at 36 days (Fig. 1). The injections do not cause the production of corpora lutea, but thecal luteinization at 26 and 36 days is obvious. The clitoris is enlarged at 16 and 26 days.

The female accessory organs are very reactive to the endogenous gonadal hormone secreted as a result of ovarian stimulation by equine gonadotropin. The oviduct responds as early as 10 days by increase in weight and epithelial height. Its percentage weight change increases until 26 days, after which it drops. Some degree of reactivity in the uterus is apparent histologically at 10 days. At 16 days a sudden peak is reached in the weight of the uterus of the injected animal, accompanied by great stimulation

of the uterine glands and mucosa. The percentage increase in weight drops gradually and consistently for the next 40 days.

The testis is stimulated in its growth by equine gonadotropin as early as 6 days. After the first age there are evidences from the histological condition and increase in weight of the male accessory glands that the testis is stimulated to increase its production of male hormone. The reactivity of the gonad to this dose of gonadotropic hormone is highest at 16 days and decreases gradually to become absent by 36 days. Tubule diameter and lumen size are increased at 16 and 26 days. Interstitial tissue seems somewhat increased at 26 and 36 days.

The male accessory glands reach their maximum reactivity at 16 days, as determined by their weight increase and histological stimulation. In the ventral prostate and coagulating gland this percentage weight increase drops rather suddenly after 16 days, but in the seminal vesicle the same increase in weight is preserved in all the subsequent ages. With gonadotropic treatment the seminal vesicles acquire precocious light areas in the epithelium as early as 16 days, although these are very small and indistinct. The penis is enlarged in all injected animals after 6 days of age.

Testosterone propionate.—With this hormone, the ovary is slightly reduced in weight at all ages until 26 days, when the reduction becomes more pronounced. Testosterone propionate has no histological effect on the ovary at any age studied.

The female accessory organs are stimulated in growth in the first three ages, and after that they are apparently reduced in size. Evidence of histological stimulation is also present during the first three ages and absent in the older animals. Uterine glands are produced

precociously at 6 days by the injection of testosterone propionate and are highly stimulated at 10 and 16 days. The clitoris is greatly enlarged with male hormone at all ages.

In the male, testosterone propionate reduces the testis slightly at the younger ages and more markedly at 26 days. Histological differences occur only at this age, when there is inhibition of the formation of lumina and reduction in the diameter of the tubules.

The male accessory glands all reach their maximum reactivity to male hormone at the age of 16 days. Light areas are precociously produced in the seminal vesicle at 16 days. The penis is greatly enlarged at all ages with male hormone.

Estradiol benzoate.—In the dose used, this hormone has little or no effect on the growth and histology of the ovary at any age studied. The female accessory organs respond to this dose of female hormone with a very high degree of stimulation in growth and development at the ages of 6 and 10 days, decreasing gradually in older ages. Uterine glands are produced precociously at 6 days, and highly stimulated at 10 and 16 days. The clitoris is greatly enlarged until 26 days. The genital region is highly swollen at 6 and 10 days. The vagina is precociously opened at 6 days, and the smear of the injected animals at the younger ages is composed of cornified and epithelial cells.

The testis is reduced in weight consistently after 6 days, although no inhibition can be detected histologically. The production of male hormone is inhibited to some degree, since accessory organs are somewhat reduced in weight at certain ages, and the development of light areas in the ventral prostate at 16 days is inhibited completely and the penis reduced in size at 10 and 16 days of age.

DISCUSSION

The rapidity of development observed in the reproductive system of the hamster during embryonic life (Graves, 1945; Ortiz, 1945) is evident in the postnatal development of this system in only some respects. While differentiation occurs more rapidly in certain phases of development in some organs, in others it occurs at approximately the same speed, or even at a slower rate than in the rat or the mouse.

The hamster ovary serves as an example of a rapidly maturing organ. It is far less developed at birth than is that of the rat; but, in spite of this, ovulation occurs at 30 days of age (Graves, 1945), and a fertile copulation was obtained by Selle (1945) in a female hamster at 28 days, while in the rat ovulation has been reported at ages ranging from 40 to 70 days, depending upon the strain of rats (Long and Evans, 1922; Hargitt, 1930; Blandau and Money, 1943).

A peculiar characteristic in the early maturation of the hamster ovary is that the rapidity of development is limited to a short period just before ovulation. Follicular development is very slow, and antra are only beginning to form in some follicles at 26 days of age, as reported in the present work. This delayed formation of antra is in sharp contrast to the condition in the rat, in which follicular antra are present at 10 days (Price and Ortiz, 1944) and 11 days (Hargitt, 1930), and in the mouse, in which antra have been reported at 12–15 days (Engle, 1931). There is evidently in the hamster ovary a sudden and marked growth in some of the follicles after 26 days of age, resulting in ovulation within a few days, while the great majority of the follicles remain small and undeveloped (Fig. 2). This corroborates the findings of Deanesly (1938), who reported that antra appear just before ovulation.

The large number of polyovular follicles is a characteristic of the hamster ovary. Although these follicles have been reported in the rat (Lane, 1938), they are very rare in this animal but are quite common in the opossum, dog, cat, and some other mammals (Hartman, 1926). As in all these animals, polyovular follicles are more numerous in the ovaries at younger ages. They degenerate gradually and disappear almost completely before puberty.

The beginning of oestrous cycles in the hamster is closely associated with the first ovulation, as is the case in the rat. The first spontaneous oestrus in our hamster colony was observed around 30 days. Peczenik (1942) reports the first oestrus in his colony at 32 days of age, and Selle (1945) in one female, at 28 days. However, vaginal opening in the hamster is not a phenomenon that occurs as ovulation and oestrus cycles begin but is extremely precocious. In the normal females studied, the vagina opened at approximately 10 days of age, which coincides with the observations of Bond (1945) and Kupperman, Greenblatt, and Hair (1944). In the rat the opening of the vagina occurs around 50 days of age (Wade and Haselwood, 1941; Blandeau and Money, 1943). Therefore, while in the rat vaginal introitus can be considered as one of the signs of sexual maturity, in the hamster it is not linked with the maturity phenomena. This precocity of vaginal opening in the hamster, however, is not associated with early differentiation of the uterus, for the rate of development of the hamster and the rat uteri is about the same. Endometrial glands begin to form in the uterus of the rat (Price and Ortiz, 1944) and the hamster at 10 days of age.

In the male the rate of development of the reproductive tract of the hamster and the rat coincide more closely than in the

female. The testis develops in both animals at almost the same speed. At birth and at 6 days the histological condition of the gonad is very similar, and the stages of differentiation which follow occur at approximately the same ages. According to Hargitt (1926), in the rat at 15 days the testis tubules begin to develop lumina; at 23 days testis descent begins, and at 38 days sperm heads appear for the first time. Testis tubules in the hamster begin to develop lumina at 16 days, testis descent starts at 26, and the first sperm heads are observed at 36 days of age. Since at this age sperm heads were not present in the testes of all animals studied, it is assumed that they are just beginning to develop and that spermatozoa are not mature and ready for fertilization until several days later. Bond (1945) did not obtain fertile copulations in males until 43 days of age.

The development of the ventral prostate gland takes place at about the same rate in the rat and the hamster. Light areas are found in the epithelium of the rat prostate at 12 days, and the gland reaches the adult histological condition before the third week of life (Price, 1936). In the hamster, light areas first appear at 16 days, and by 26 days the gland is very well developed histologically. The seminal vesicle, however, acquires the adult histological condition with cellular light areas in the hamster at 26 days, while, in the rat, secretion granules do not develop until 35 days of age (Price, 1936), so that the mature condition in the rat seminal vesicle is delayed as compared to the hamster.

An outstanding characteristic in the development of the reproductive system of the hamster is the sudden acceleration of growth of almost all the organs between 16 and 26 days of age (Table 1). Since this acceleration in organ growth is not accompanied by similar accelera-

tion in body growth, it seems logical to assume that there is either a sudden increase in organ reactivity to hormone stimulation or a sudden increase in production or release of gonadotropic hormone from the hypophysis in the normal animal at this age, which stimulates the gonads to increase in weight and in hormone production and thus affects the accessory organs. Although no work has yet been reported on assays of anterior lobe hormone content in the hamster, the work of Clark (1935) on implants of rat hypophyses assayed in young mice shows that there is a sharp rise in gonadotropic hormone content at between 13 and 20 days in the female rat.

A comparison of the effects of hormone injections in the hamster and the rat (Price and Ortiz, 1944) establishes two points: (1) in general, the reproductive system of the hamster is much less responsive than that of the rat to the same hormone doses, and (2) the period of highest reactivity occurs earlier in the hamster than in the rat. The percentage weight changes are greater in the rat in all organs with all three injected hormones; but the difference is even more marked with the gonadotropin. The greatest reactivity, as determined by percentage weight increase, is reached in the rat at 26 days with all organs except the testis, while in the hamster it occurs at 16 days or earlier in all except the ovary, which reaches its peak at 36 days.

The delayed reactivity of the ovary may be explained on the basis of the fact that follicular antra are not present in the hamster ovary until about 26 days of age, and gonadotropic hormones do not affect follicles to a great extent before antra are formed (Hertz and Hisaw, 1934). As soon as antra are present in the hamster ovary, the degree of reactivity to gonadotropic stimulation rises rapidly above that at the previous age and

reaches the 36-day peak. Peczenik (1942) also found the hamster ovary most reactive to gonadotropic hormones at 30 days of age.

The loss of reactivity in the hamster ovary after 36 days is similar to that found in the rat ovary after 26 days (Price and Ortiz, 1944). While the ovary of the 56-day-old hamster injected with gonadotropin resembles that at 36 days, in the extreme enlargement of the follicles and their hemorrhagic condition, the organ shows less percentage increase in weight at this age over the normal than it does at 36 days, so that an actual loss in reactivity occurs.

The failure of production of corpora lutea in the ovaries at 26 and 36 days with gonadotropic treatment is another instance of lower reactivity in the hamster as compared with the rat, since the same dose of equine gonadotropin produced corpora lutea precociously in the rat ovaries at these ages (Price and Ortiz, 1944). The daily dose of 10 r.u. (20 IU) for 6 days produced thecal luteinization but was too low to stimulate follicular luteinization in the hamster ovary; Peczenik (1942) was able to produce some luteinization of the stroma and follicles in 24-day-old hamsters with daily doses of 200 IU of chorionic gonadotropin given for 12 days, and the formation of massive corpora lutea with anterior pituitary extract. Similar findings have been reported in the immature guinea pig by Leathem and Starkey (1940). They obtained with 5 r.u. of PMS the same results in the guinea-pig ovary that were obtained in the hamster in the present work—great follicular stimulation, no corpora lutea, and some thecal luteinization. However, with higher doses or longer periods of injection, corpora lutea were produced.

The increase in production of female hormone in the ovaries under gonado-

tropic stimulation, as judged by changes in weight and histology of the uterus and oviduct, begins at 10 days in the hamster, as in the rat (Price and Ortiz, 1944), and is evident at all the ages thereafter. Androgenic hormone is also produced under gonadotropic stimulation by 16 days of age, since the clitoris was enlarged in the injected females. This was previously reported in the hamster by Peczenik (1942) and in the rat by several investigators (Bradbury and Gaensbauer, 1939; Marx and Bradbury, 1940; Price and Ortiz, 1944; and others).

Injection of testosterone propionate and estradiol benzoate produces some inhibition of growth in the ovaries at all ages, which is slightly accentuated at 26 days. However, the doses of sex hormones used in the present experiments do not produce any detectable histological changes, while in the rat the same cases caused inhibition or stimulation of follicular growth, depending upon the age (Price and Ortiz, 1944). However, when the hamster ovary was subjected to chronic extrinization with pellets of diethylstilbestrol for $3\frac{1}{2}$ – $8\frac{1}{2}$ months, beginning at 40 days, it was completely suppressed in its activity and consisted only of interstitial cells and a few small follicles (Koneff, Simpson, and Evans, 1946).

The oviduct and uterus are very responsive to gonadal hormone produced by injection of gonadotropin and to estradiol benzoate and less reactive to testosterone propionate, as can be seen from the percentage changes in weight. This was also true in the rat (Price and Ortiz, 1944). One of the most outstanding characteristics of the female accessory reproductive organs is that they show their highest reactivity to female hormone, as judged by weight increase, at 6 and 10 days of age. Percentage weight increase declines gradually as the

animal grows older. This is not the case in the rat, in which the responsiveness of the oviduct and uterus increases gradually, reaches its peak at 26 days, and drops thereafter (Price and Ortiz, 1944).

The oviduct and uterus at 26 and 36 days are still reactive to female hormone produced by gonadotropin-stimulated ovaries, but they seem to lose their reactivity to injected female and male hormones and are even apparently reduced in weight by these hormones. Since their capacity to respond to increased amounts of female hormone is still present at these ages, as can be seen from their percentage weight increase with gonadotropic treatment, it can be assumed that the doses of sex hormones are too low to stimulate these organs at these ages. Therefore, the apparent reduction of the uterus and oviduct at 26 and 36 days with exogenous sex hormones should be interpreted as a lack of stimulation of organs which are extremely variable in size rather than as true inhibition of growth. Larger doses would probably continue to enlarge the uterus, since the implantation of pellets of diethylstilbestrol for $3\frac{1}{2}$ – $8\frac{1}{2}$ months, starting at 40 days of age, increased the uterine weight in the hamster two to four times over that of the control (Koneff, Simpson, and Evans, 1946).

The early development of great reactivity of the uterus to female hormone treatment as demonstrated by the percentage weight increase is shown also in the histological differentiation. Glands are produced precociously at 6 days of age by estradiol benzoate and stimulated at 10 and 16 days. Although a maximum weight increase is not obtained with testosterone propionate, the uterus is very sensitive to the androgen in the younger ages, as shown by precocious production of glands at 6 days, which are as well de-

veloped as in estrogen-treated females. At 10 and 16 days, glands are stimulated to the same degree by all three substances. In these respects the hamster is more responsive than the rat, for the same doses of sex hormones did not stimulate the formation of uterine glands precociously in the rat. Precocious formation of uterine glands by both androgen and estrogen treatment has been reported in the opossum by Moore and Failor (1946).

The vaginal membrane of the hamster is more sensitive to estrogen than is that of the rat, since with the same dose of estradiol benzoate the vagina of the rat did not open until 18 days (Price and Ortiz, 1944), while in the hamster it opens at 6 days of age. Large doses of estrogen in the rat open the vagina at 9 days and produce an oestrous smear (Weichert and Kerrigan, 1942). The vaginal epithelium of the hamster responds to estrogen with a smear typical of either pro-oestrus or oestrus, according to the classification of Kupperman, Greenblatt, and Hair (1944), Kent and Mixner (1945), Kent and Smith (1945), and Ward (1946).

As has already been reported by Peczenik (1942) and Bruner and Witschi (1946), the hamster clitoris is very reactive to injected androgen and estrogen. This has been found in the rat by many investigators (Turner and Burkhardt, 1939; Selye, 1940; Weichert and Kerrigan, 1942; Price and Ortiz, 1944; and others).

The reactivity of the male organs of the hamster is less than that of the rat. The weight changes produced by the hormone injections are lower, and the histological changes, in general, are less pronounced in the hamster. As in the rat (Price and Ortiz, 1944), the testis is stimulated by gonadotropin at 6 days, which is earlier than the ovary in both

animals. Although no signs of increased hormone production are detectable until 10 days in the hamster, testis growth is significantly stimulated at the first two ages and reaches its peak of reactivity at 16 days. The highest testis response in the rat is at 14 days and is higher than in the hamster. At 26 days the hamster testis is much less reactive, and by 36 days it seems to have lost its reactivity to this dose of equine gonadotropin.

The injection of male hormone inhibits the growth of the testis in all animals of 10 days or older but is most effective at 26 days. As has already been discussed, the testis has an increase in growth rate between the ages of 16 and 26 days; this seems to be also the period of greatest inhibition of testis growth and development by testosterone propionate.

Estradiol benzoate also inhibits the growth of the testis at all ages, beginning at 10 days, and inhibits the production of male hormone in animals 16 days and older, as is evidenced by weight reduction and histological changes of the male accessory organs at some ages, as well as by the reduction in the penis size. The dose given in these experiments, however, was not high enough to destroy the germinal epithelium or the interstitial tissue, but such destruction has been produced with higher doses and much longer periods of treatment (Koneff Simpson, and Evans, 1946).

The male accessory glands reach their peak of reactivity at 16 days with both gonadotropin and testosterone propionate injections. In the rat the seminal vesicle and coagulating gland are more reactive to male hormone at 26 days, as they approach puberty (Price and Ortiz, 1944); but in the mouse the maximum of stimulation of the seminal vesicle after treatment with gonadogen

was reached at 10 and 15 days of age (Bishop and Leathem, 1946).

SUMMARY

The normal development of the reproductive system of the golden hamster and the effects of hormones on this system were studied in 546 animals from birth to puberty. Results can be summarized as follows:

NORMAL DEVELOPMENT

1. The gonads and accessory reproductive organs in both sexes grow and develop slowly during the first 2 weeks after birth, but they have a sudden acceleration of growth at between 16 and 26 days.

2. Follicular antra first appear in the ovary at 26 days, the first spontaneous oestrus occurs around 30 days, and corpora lutea are present in the ovary for the first time by 36 days. The vagina opens as soon as the female reaches a body weight of approximately 8 gm., which is usually around 10 days of age.

3. The testis tubules begin to form lumina at 16 days, testes begin to descend at about 26 days, and sperm heads first appear by 36 days. The ventral prostate acquires light areas at 16 days, and the seminal vesicle at 26 days. By 26 days all the male accessory glands have reached their adult histological condition, and there is evidence of secretion in the seminal vesicle and coagulating gland.

EFFECT OF HORMONE INJECTIONS

Ten r.u. of equine gonadotropin, 0.1 mg. of testosterone propionate, and 1 r.u. of estradiol benzoate were injected subcutaneously for a period of 6 days, starting at birth and at 4, 10, 20, and 30 days of age. Animals were killed and autopsied about 24 hours after the last injection, at

the ages of 6, 10, 16, 26, and 36 days. The reactivity of the ovary, oviduct, uterus, testis, ventral prostate, seminal vesicle, and coagulating gland was determined by the changes in weight and histology as compared to the organs of the normal controls.

1. The ovary is first stimulated by gonadotropin at 10 days, when both weight and hormone production are increased. The reactivity of the ovary to gonadotropin increases at the next ages and reaches a sudden peak at 36 days, when there is great follicular stimulation and some thecal and interstitial luteinization but no formation of corpora lutea. Testosterone propionate reduces the size of the ovary, and estradiol benzoate has little or no effect on ovarian weight. The histology of the ovary is not affected by either sex hormone.

2. The oviduct and uterus are highly stimulated by ovarian hormone, with gonadotropic treatment beginning at 10 days, but reactivity gradually decreases after 16 days. They are also stimulated by testosterone propionate up to 16 days and not affected at older ages. Estradiol benzoate produces the greatest response of these organs at 6 and 10 days and has no effect after 16 days. Uterine glands are produced precociously at 6 days by testosterone propionate and estradiol benzoate and are highly stimulated in their development at 10 and 16 days with gonadotropic treatment and the injected sex hormones.

3. The vagina is opened precociously at 6 days with estradiol benzoate and an oestrous or pro-oestrous smear is produced.

4. The testis is stimulated in growth with gonadotropin at all ages. Beginning at 10 days, there are evidences from the increase in weight and changes in histological condition of the male accessory glands that the testis is producing an in-

creased amount of male hormone under the gonadotropic treatment. The reactivity of the gonad to this dose of equine gonadotropin is highest at 16 days, decreases gradually thereafter, and is absent at 36 days. Interstitial tissue is slightly increased at 26 and 36 days. Testosterone propionate inhibits the testis slightly at the younger ages and more markedly at 26 days. Estradiol benzoate reduces the weight and the male hormone production in the testis slightly.

5. The ventral prostate, seminal vesicle, and coagulating gland are stimulated by gonadotropic treatment and by testosterone propionate and reach their peak of reactivity at 16 days. Light areas in the seminal vesicle are produced precociously at 16 days with gonadal hormone and testosterone propionate. Estradiol benzoate reduces the growth of the male accessory glands at some ages and inhibits the light areas in the prostate at 16 days.

CONCLUSIONS

1. The hamster reproductive system does not continue its rapid prenatal growth in postnatal life except in the early maturation of the ovary, the establishment of the oestrous cycles, and the extremely precocious opening of the vagina.

2. The reproductive system of the hamster is, in general, less reactive than that of the rat to the same hormone doses.

3. The age of highest reactivity of the male organs is 16 days, of the ovary 36 days, and of the female accessory organs 6 and 10 days.

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PLATE I

FIG. 1.—Ovary of a 36-day-old hamster injected with equine gonadotropin. Compare with Fig. 2 and note the stimulation of the follicles. $\times 30$.

FIG. 2.—Ovary of a normal 36-day-old hamster, littermate to female of Fig. 1. Note corpus luteum forming at upper right. $\times 30$.

FIG. 3.—Seminal vesicle of a normal 36-day-old hamster, showing light areas. $\times 340$.

FIG. 4.—Uterus of a normal 6-day-old hamster. $\times 27$.

FIG. 5.—Uterus of a 6-day-old hamster injected with estradiol benzoate. Compare with Fig. 4 and note stimulation of mucosa and endometrium and precocious gland development. $\times 27$.

